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Ryegrass seed viability in function of phenological stage of herbicides application[1]

Viabilidade de sementes de azevém em função do estádio fenológico de aplicação de herbicidas

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Abstract - Ryegrass is an important weed in maize crops and winter cereals. The emergence of glyphosate-resistant biotypes, the main herbicide used in the Brazilian agricultural system, and the ability of natural reseeding, have raised the need to seek alternatives to minimize seed bank replenishment of this species. The objective of this study was to evaluate the effect of herbicide application at different growth stages of glyphosate-sensitive ryegrass plants on the viability of seeds from these plants. To this end, ryegrass plants were submitted to the application of glyphosate and clethodim association (750 g a.e. ha^{-1} + 120 g a.i. ha^{-1}, respectively) in 10 in different development stages. The seeds produced were evaluated for their viability in tetrazolium test and germination in a B.O.D (Biochemical Oxygen Demand)-type growth chamber, with subsequent tetrazolium test and plants emergence in peat substrate. The results indicate that desiccation time limit for ryegrass to not produce viable seeds is the stage prior to complete anthesis, and at this stage there was complete reduction of viability, germination and emergence of ryegrass seeds exposed to the application of glyphosate + clethodim association.
Keywords: seed bank; phenology; clethodim; glyphosate; *Lolium multiflorum*

Resumo - O azevém é uma importante planta daninha na cultura do milho e em cereais de inverno. O surgimento de biótipos resistentes ao glyphosate, principal herbicida utilizado no sistema agrícola brasileiro, e a capacidade de ressemeadura natural, tem-se suscitado a necessidade de buscar alternativas para minimizar o reabastecimento do banco de sementes dessa espécie. O objetivo deste trabalho foi avaliar o efeito da aplicação de herbicidas em diferentes estádios fenológicos de plantas de azevém sensível ao glyphosate, sobre a viabilidade das sementes originadas dessas plantas. Para tal, plantas de azevém foram submetidas à aplicação da associação dos herbicidas glyphosate e clethodim (750 g e.a. ha^{-1} + 120g i.a. ha^{-1}, respectivamente) em 10 diferentes estádios de desenvolvimento. As sementes produzidas foram avaliadas quanto a sua viabilidade em teste de tetrazólio; germinação em câmara de crescimento tipo B.O.D. com posterior teste de tetrazólio e emergência de plantas em substrato de turfa. Os resultados indicam que a época limite de dessecação do azevém para que não produza sementes viáveis é o estádio anterior à antese

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completa, sendo que neste estádio ocorreu a redução total da viabilidade, germinação e emergência das sementes de azevém expostas a aplicação da associação de glyphosate + clethodim.
Palavras-chaves: banco de sementes; fenologia; clethodim; glyphosate; *Lolium multiflorum*

Introduction

Ryegrass (*Lolium multiflorum* Lam.) is an important wintry forage, with a high biomass production, high tolerance to grazing and excess moisture (Boldrini et al., 2008). The species is widely found in the Brazilian state of Rio Grande do Sul, used as forage for animal feed, as well as winter cover and straw supply to the direct seeding system (Christoffoleti and López-Ovejero, 2003). Together with this, they are relevant weed plants, mainly for maize crops and winter cereals in general.

Ryegrass is a cross-pollination self-incompatible species, with its pollen spread by the wind. In late spring, ryegrass plants bloom and bear fruit in abundance and, after physiological maturity, seeds abscission occurs which, if not harvested in time, will settle on the ground (Piana et al., 1986). The potential for seed production in some ryegrass biotypes can reach about 3500 seeds per plant (Galvan et al., 2011). Natural reseeding is useful when the purpose is forage production; however, for grain production, it causes control problems due to different emergence flows, and plants occur in different developments (Piana et al., 1986; Galli et al., 2005).

In the direct seeding system or in orchards, its control is usually performed with the application of non-selective herbicides in different phenological stages, and glyphosate is the most widely used herbicide for this purpose (Christoffoleti and López-Ovejero, 2003).

Management effectiveness of weeds present in a given area is key to future population dynamics, given that plants that are not eliminated finalize their cycle, have their seeds scattered at different distances and thus replenish the soil seed bank. Although genetically defined for each species, the seeds chemical composition may undergo changes imposed by environmental conditions during the development period, so that the higher the seed stores content, the greater the originating seedling vigor (Garcia et al., 2007).

Given the importance of ryegrass as weed and its ability to survive various environmental conditions, it is critical to know the plant developmental stage where the stress caused by the application of herbicides prevents or reduces the production of viable seeds with the aim of reducing the seed bank in the soil. To that end, the aim of this study was to evaluate the effect of herbicides association applied at different growth stages of ryegrass plants on the viability of the seeds produced.

Material and Methods

The study was developed at the Centro de Extensão e Pesquisa Agropecuária [Cepagro (Extension and Agricultural Research Center)] at Agronomy and Veterinary Medicine Faculty of the University of Passo Fundo, Passo Fundo, RS, Brazil, located in geographical coordinates latitude 28° 13’ 01” S, longitude 52° 23’ 37” W and approximate altitude of 700 m above the sea level. The climate in the area, according to Köppen classification, is humid subtropical (Cfa). The material studied was ryegrass seeds collected from glyphosate-sensitive biotypes and the experiment was conducted in the period May to October 2011.

The ryegrass seeds were sown in plastic pots with a volumetric capacity of 5 L each, filled with typical dark red latosol-type soil. In each experimental unit, four plants were cultivated, and during the seed production period the pots were irrigated according to the plants water requirements.

Ryegrass plants were treated with 750 g a.e. ha^{-1} of glyphosate herbicide (Zapp Qi 620, Syngenta) + 120 g a.i. ha^{-1} of clethodim (Select 240 EC, Arysta) in 10 different growth stages, based on the scales by Zadoks et al. (1974) and

Hess et al. (1997). The phenological stages were: 1 = flag leaf sheath extending; 2 = first spikelet of inflorescence just visible; 3 = beginning on anthesis; 4 = anthesis in 50%; 5 = anthesis completed; 6 = juicy caryopsis; 7 = medium milky grain; 8 = soft dough grain; 9 = hard caryopsis; and 10 = maturation with dry straw.

The herbicides applications were carried out by a precision knapsack sprayer, XR 11002 flat jet nozzle tips, with an application volume of 200 L ha^{-1} in environmental conditions that were favorable to the application. At the time of application, temperatures ranged from 18 to 20 °C, air humidity of 70 to 80%, and absence of wind. The applications were sequenced according to ryegrass development stage.

After plants death, about 30 days after herbicide application, the spikes were collected and the seeds formed were removed from them. These were stored and kept refrigerated at temperatures close to 0 °C for three days in order to overcome the seeds dormancy. Subsequently, the following variables were evaluated: germination test, seed viability in tetrazolium and seedlings emergence in substrate in a greenhouse.

Germination test: to evaluate the germination vigor of ryegrass seeds, an experiment was conducted in a randomized block design with four replications of 50 seeds each. First the seeds underwent sterilization and disinfection by exposure for 5 minutes in 70% alcohol and 20 minutes in 2% sodium hypochlorite after being triply washed in running water. Then they were exposed to 0.2% potassium nitrate for 4 hours to help overcome dormancy. Afterwards, the seeds were placed in gerbox boxes containing moistened germination paper in water/paper ratio 2.5 times the paper weight, and re-dampened when needed. The material was stored in a B.O.D. (Biochemical Oxygen Demand)-type growth chamber at 20 °C and a 12 hours photoperiod with light-incidence of approximately 10.9 K Lux. The evaluations were performed at 7 and 14 days after sowing. The seeds with visible extrusion of the radicle or coleoptile were considered as germinated, according to the Regras para Análise de Sementes [RAS (Rules for Seed Analysis)] (Brasil, 2009). The seeds that did not germinate in the chamber were submitted to the tetrazolium test.

Tetrazolium test: To check the feasibility of ryegrass seeds, an experiment with four replications of 50 seeds each was conducted in a completely randomized design. Single seeds which remained dormant in the end of the germination test and seeds which had not undergone the germination test were used to complete the number of experimental units. After sterilization, the seeds were placed in gerbox boxes between two germination paper sheets moistened in the water/paper ratio of 2.5. The seeds were kept in this condition for a period of 24 hours to promote the seeds hydration.

Then, the seeds were longitudinally cut with a scalpel before being placed in contact with the tetrazolium solution at a concentration of 0.5%, for 6 hours at 30 °C temperature. Later, the seeds were evaluated for their viability, according to Brasil (2009).

Finally, the emergence of ryegrass seedlings from seeds germinated in pots filled with peat substrate in a greenhouse was evaluated. The experiment was conducted in a completely randomized design with 4 repetitions. For this, 10 ryegrass seeds were placed per pot, with a volumetric capacity of 1 L. Emergence assessments of ryegrass plants took place weekly for 28 days, totaling four weeks. The results obtained were subjected to analysis of variance and the means compared by Tukey's test at 5% error probability.

Results and Discussion

From the results of analysis of variance, significant effect was observed at the time of the herbicides application in different stages of development for the viability of seeds produced by ryegrass plant.

Ryegrass seeds were shown to be viable to tetrazolium test from the completed anthesis stage. However, with no high averages, which did not differ from previous stages, such as the flag leaf sheath extending, first spikelet of inflorescence just visible, beginning on anthesis and anthesis in 50%. Nonetheless, maximum viability was found in seeds from stages soft dough grain, hard caryopsis and maturation with dry straw.

For seeds submitted to germination test in a growth chamber with subsequent tetrazolium test, the existence of suitable seeds from the juicy caryopsis stage was verified; however, the highest numerical value corresponds to the maturation with dry straw stage, which statistically did not differ from the soft dough grain and hard caryopsis stages (Table 1).

Table 1. Tetrazolium test (%) germination in growth chamber (%) and seeds emergence (%) of ryegrass depending on the application of glyphosate + clethodim in different growth stages of ryegrass plants development. Passo Fundo, RS – 2011.

Herbicide application timing	Tetrazolium test	Germination in growth chamber	Emergence in a pot
Flag leaf sheath extending	0.0 c[1]	0.0 d	0.0 c[1]
First spikelet of inflorescence just visible	0.0 c	0.0 d	0.0 c
Beginning on anthesis	0.0 c	0.0 d	0.0 c
Anthesis in 50%	0.0 c	0.0 d	0.0 c
Anthesis completed	10.0 c	0.0 d	0.0 c
Juicy caryopsis	35.0 b	22.5 c	0.0 c
Medium milky grain	47.5 b	50.0 b	2.50 c
Soft dough grain	87.5 a	76.2 a	15.0 c
Hard caryopsis	90.0 a	77.5 a	32.5 b
Maturation with dry straw	97.5 a	90.0 a	80.0 a
C.V. (%)	15.0	19.7	35.8

[1]Means followed by the same letter in the column do not differ by Tukey's test ($P < 0.05$).

The lack of viability in the completed anthesis stage in the germination test followed by tetrazolium test is primarily due to the microclimate, brought about by moisture, existing in the gerbox, providing that less vigorous seeds begin to putrefy during the incubation period. Contrary to what happened in the evaluation in the tetrazolium test.

Larcher (2006) states that plants without vigor, senescent or stressed by the environment produce eggs that are underdeveloped or incapable of developing seeds and therefore normal seedlings. Thus, the herbicide may have caused stress on the plant which, in the natural order to perpetuate the species, produced seeds; however, these were not fully developed.

When the seeds were evaluated for their behavior in pots, seedling emergence was obtained only when desiccation was carried out from the medium milky grain stage, in a nonsignificant amount (Table 1). The stage with the highest emergence was maturation with dry straw, being statistically superior to the other seasons, providing 2.5 times the stage immediately below, hard caryopsis.

The difference observed between emergence of plants in pots and seed viability in the tetrazolium test, with or without germination in a growth chamber, is due especially to the fact that the seeds did not have a full physiological process, and although the embryo was viable, the seed did not have sufficient reserves to enable withstanding adverse environmental conditions, nor energy to break through the layers of soil to reach the surface.

Each stage of plant development has an effect on the others, so that the physiological and nutritional states of the mother plant affects the amount of nutrients available to the translocation to the seeds (Larcher, 2006).

Water stress, for example, can accelerate flowering to ensure perpetuation, but there is less production of flowers and seeds (Monquero and Christoffoleti, 2005).

Garcia et al. (2007) have noted in their work that the stress caused by irrigation with saline water and increased soil salinity has decreased contents of crude protein, ether extract, mineral matter, total carbohydrates and seeds dry matter, final germination percentage on paper, percentage of sand germination, germination speed index, aerial parts fresh and dry matter of maize seedlings followed by the increase in the percentage of damaged seeds and abnormal seedlings.

Bezerra et al. (2010), in turn, have observed greater production of flowers of sesame plants with increasing water stress levels compared to that of plants grown in soil under field capacity conditions; however, the number of fruits/plant, as well as production measured as mass of fruits/plant and seed/plant decreased with increasing water stress.

Seed size, embryo differentiation stage and the nutrients reserve decisively influence germination capacity and vigor (Larcher, 2006). In this context, an event seemingly with little influence on the seeds can take on great importance in viability and germination vigor (Larcher, 2006).

On the other hand, according to Galvan et al. (2011), the production of ryegrass seeds is high, approximately 3,500 seeds per plant. Thus, while in a state of reduced seed germination and seedling emergence, as seen in the medium milky grain stage, this may be sufficient to maintain the species in the area.

Still, it should be noted that the emergence of ryegrass in the field takes place in stages, allowing the occurrence of plants at different stages of development at the same time in the same area, and, together with this, the plant flowering process is also uneven, being possible that in the same plant dry spikes and other ones not flowering occur. Hampton & Hebbletwaite (1982) mention that ryegrass is a crop that is highly uneven in maturation between and within the spikes.

Thus, the lack of planning for desiccation of agricultural areas allows the seed bank to be constantly renewed in crops, in order to maintain high weed populations.

Conclusions

The time limit for application of the glyphosate + clethodim association to prevent the production of viable seeds is the completed anthesis stage. In addition, ryegrass plants emergence is possible only with seeds from the application of herbicides after the medium milky grain stage.

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